Clinical Science and Molecular Medicine (1977) 52, 241-248.

Lysosomal changes in liver tissue from patients with the Dubin–Johnson–Sprinz syndrome

CAROL A. SEYMOUR, G. NEALE AND T. J. PETERS
Department of Medicine, Royal Postgraduate Medical School, London

(Received 19 August 1975; accepted 27 August 1976)

Summary

1. Clinical, morphological and biochemical data, including data obtained from the application of subcellular fractionation techniques to liver biopsy specimens, are presented for two patients with the Dubin–Johnson–Sprinz (DJS) syndrome.

2. Subcellular fractionation experiments demonstrate that the lysosomes, which have strikingly reduced equilibrium densities, accumulate melanin. Morphological studies confirm the presence of pigments within lysosomes.

3. Although there are increased activities of lysosomal acid hydrolases in the liver tissue from patients with the DJS syndrome, the integrity of these organelles is essentially normal and therefore the accumulation of pigment would not be expected to initiate liver damage. The DJS syndrome is thus a benign type of secondary lysosomal storage disease.

Key words: Dubin–Johnson–Sprinz syndrome, lipofuscin, liver, lysosomes, melanin, needle biopsy, subcellular fractionation.

Abbreviations: DJS syndrome, Dubin–Johnson–Sprinz syndrome; EDTA, ethylene diaminetetra-acetate.

Introduction

The genetic syndrome of chronic idiopathic hyperbilirubinaemia associated with a lipochrome-like pigment in hepatocytes was described independently by Dubin & Johnson (1954) and Sprinz & Nelson (1954). The syndrome is often undiagnosed until environmental factors or intercurrent illness accentuate, often misleadingly, the hyperbilirubinaemia (Cohen, Lewis & Arias, 1972; Schinella, 1972; Edwards, 1975). Although the disease is lifelong, the prognosis is excellent (Dubin, 1958).

Despite the black–green appearance noted in the liver tissue macroscopically, microscopic examination reveals brown pigment granules within the hepatocyte. The nature of this amorphous brown pigment was uncertain when first described (Dubin & Johnson, 1954; Sprinz & Nelson, 1954). More recently there is histochemical and electron-microscope evidence that lipofuscin and melanin are the pigments which accumulate within the liver tissue, and particularly within the hepatic lysosomes (Barone, Inferrara & Carrozza, 1969; Arias, 1973).

In this paper we describe the results of applying analytical subcellular fractionation techniques to liver tissue from two patients with the Dubin–Johnson–Sprinz syndrome to study the properties of their hepatic lysosomes.

Case histories

Patient 1

B.A., a 50-year-old male from Mauritius, was admitted to hospital with chest pain and malaise. There was no family history of jaundice. He drank alcohol only in moderation and there was no past history of hepatitis,
of drug addiction, of blood transfusions or of exposure to hepatotoxins. Examination revealed a pale, icteric man with no other skin stigmata of liver disease but with the signs of pericarditis.

Haematological findings were normal with no evidence of haemolysis. The serum bilirubin was 52.7 mmol/l (normal 2-14 mmol/l) with 81% (normal 0-15%) in the conjugated form. Urine contained bilirubin, but the urine coproporphyrin I:III ratio was normal, in contrast to a report by Ben-Ezzer, Blonder, Shani, Seligsohn, Post, Adam & Szeinberg (1973).

Other liver functions were normal except for the clearance of bromosulphthalein, which showed a secondary rise of plasma concentration of the dye to 58% at 2 h. This suggested the diagnosis of the Dubin-Johnson-Sprinz syndrome. The liver was normal in size on the radio-colloid gamma-scan and liver biopsy obtained with a Menghini needle showed the typical black-green tissue.

The patient’s convalescence from viral pericarditis was complicated by the development of pleural and pericardial effusions. After 3 weeks in hospital he was discharged well but with persistent scleral icterus.

Patient 2

D.C., a 36-year-old schoolmistress, first presented with jaundice and episodes of passing dark urine, 1 year after commencing taking the oral contraceptive Minilyn [lynoestrenol (2.5 mg)/ethinyloestradiol (0.05 mg); Organon Laboratories Ltd]. She was routinely attending a hospital out-patient clinic with mild chronic renal disease which followed an episode of tonsillitis 4 years previously. The renal disease, which was clinically and histologically consistent with a minimal change glomerulonephritis, had responded to steroids.

There was no family history of relevance and she took alcohol only occasionally. There was no recent exposure to drugs nor to hepatotoxins (steroids had been discontinued 3 years before the present symptoms occurred). Examination showed a healthy, mildly icteric woman with no other dermatological stigmata of liver disease.

Haematological investigations were normal and there was no evidence of haemolysis. The serum bilirubin was 32.3 mmol/l, of which 83% was conjugated. Cholecystography failed to opacify the gall bladder and a bromosulphthalein clearance was abnormal with a delayed rise (20%) at 2 h. Urine analysis showed the presence of bilirubin but no excess of urobilinogen and the ratio of urinary coproporphyrin I:coproporphyrin III was normal. Other biochemical tests were also normal. Liver biopsy showed the classical features of the DJS syndrome.

Neither patient 1 nor patient 2 gave a family history of jaundice.

Methods

Liver tissue was obtained by needle biopsy with a Menghini needle. The tissue was taken into ice-cold sucrose solution (0.25 mol/l) containing disodium EDTA (1 mmol/l), pH 7.2, and ethanol (20 mmol/l), and divided into three portions. One portion was processed for routine histological examination and another was processed for electron microscopy. The remainder was disrupted in a Dounce homogenizer with a loose-fitting pestle. Total lysosomal enzyme activities (Seymour & Peters, 1977) and the integrity of the lysosomes as reflected by the latent and sedimentable N-acetyl-β-glucosaminidase were determined as described by Peters, Heath, Wansbrough-Jones & Doe (1975). Subcellular fractionation of the tissue extracts was performed as described by Peters, Müller & de Duve (1971) and Seymour, Neale & Peters (1974). Enzyme activities were expressed as munits/mg of protein in the liver homogenates, where 1 unit is equal to 1 µmol of substrate transformed/min. Proteins were assayed by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as a standard. Melanin was assayed fluorimetrically by the method of Rosenthal, Kreider & Shiman (1973).

Urine coproporphyrins were measured as described by Koskela, Toivonen & Aldercreutz (1967).

Results

Both liver biopsies were macroscopically dark brown–green but under the light-microscope a variable brown granular pigmentation in the hepatocytes was seen (Fig. 1). At a higher
Liver lysosomes in Dubin–Johnson–Sprinz syndrome

Fig. 1. Liver biopsy section (× 360) stained with Haematoxylin and Eosin. The arrow indicates granules within the hepatocyte.

(Facing p. 242)
Fig. 2. Electron micrograph of hepatocyte from patient D.C. showing lysosomes near the pericanalicular region, containing material of variable electron density.

Fig. 3. Electron micrograph of hepatocyte from patient D.C. showing lysosomes containing particulate material of various electron densities and contained with a unit membrane.
magnification pigment granules were noted in both parenchymal and Kupffer cells.

Fig. 2 shows an electron micrograph of a hepatocyte. Electron-dense material is present as aggregates of fine granules and globular material of medium electron density. At higher power (Fig. 3) the material is seen to be enclosed within a unit membrane characteristic of lysosomes (Essner & Novikoff, 1960).

Table 1 shows the lysosomal enzyme activities in whole homogenates of liver tissue. In the DJS syndrome, all acid hydrolase activities were increased above those found in liver biopsies from a control group (patients with normal liver function and normal liver histology). The latent N-acetyl-β-glucosaminidase was slightly reduced in one patient (B.A.) but was within normal limits for the other patient (D.J.).

The results of analytical subcellular fractionation studies are shown in Fig. 4 and Fig. 5. Fig. 4 shows the distribution of some lysosomal enzymes in patient D.C., compared with the distribution in control liver tissue from a subject of the same age.

In the control subject there was a small amount of acid hydrolase activity remaining in the sample layer, but activity mainly sedimented into the gradient with broad peaks of activity extending over the density range 1·13–1·19. In patient D.C., most acid hydrolase activity remained with the sample layer and in the lighter part of the sucrose gradient. Melanin had a similar distribution to the lysosomal marker enzymes, which suggests that melanin was contained within this organelle. (Most of the protein remained in the sample layer with a small peak of particulate activity corresponding to the mitochondria; see Fig. 5.) Identical results were obtained when liver from patient B.A. was similarly processed.

Fig. 5 shows the distribution of marker enzymes for the principal organelles of the 20 000 g pellet isolated from patient B.A. compared with the distribution in control liver tissue. In the control subject, marker enzyme profiles showed clear separation of peroxisomes (catalase, modal density 1·24) and mitochondria (malate dehydrogenase, model density 1·19). N-Acetyl-β-glucosaminidase, the lysosomal marker enzyme, was distributed in a broad peak with a modal density of 1·19, with very little activity remaining in the sample layer. Most of the protein, sedimenting into the gradient, was associated with the mitochondria.

In the DJS syndrome there was no change in the distribution of peroxisomes or mitochondria. However, striking differences were seen in the lysosomal distribution, with particulate activity remaining in the low-density region of the gradient. In addition similarity in the distribution of N-acetyl-β-glucosaminidase and melanin indicated that this pigment was contained within the lysosomes.

Discussion

The two patients described conformed to the accepted criteria for a diagnosis of the Dubin–Johnson–Sprinz syndrome. The features included a chronic intermittent conjugated hyperbilirubinaemia of variable severity associated with a characteristically abnormal retention of bromosulphthalein in the serum. The defect in excretion is also found for bilirubin, organic anions and radiographic dyes (Dubin, 1958) in subjects with otherwise normal liver function (Dubin & Johnson, 1954; Dubin, 1958; Arias, 1961). The clinical diagnosis was supported by the dark colour of the liver biopsy (Dubin & Johnson, 1954), which was shown to be due to the accumulation of brown pigment within granules (Essner & Novikoff, 1960; Toker & Trevino, 1965; Baba & Ruppert, 1972). More recently it has been claimed that the ratio of urinary uroporphyrin I: uroporphyrin III (normally 1:4) is significantly increased (to 1:9) in patients with the DJS syndrome (Ben-Ezzer et al., 1973). This increase in the ratio did not appear to be present in the two patients in this report but we do not think that this vitiates the diagnosis.

Attempts to identify the pigment by histochemical methods indicated the presence of melanin and lipofuscin (Pearse, 1961; Masuda, Taki, Nonomura & Ueda, 1962; Novikoff & Essner, 1962; Toker & Trevino, 1965; Goldfischer, Villaverde & Forschirm, 1966). We have recently used a highly sensitive technique (Rosenthal et al., 1973) to assay melanin in the small amount of liver tissue obtained from a Menghini needle. Excitation and fluorescent maxima for tissue melanin derivatives were identical with those from a sample of synthetic melanin (Swan & Waggot, 1970). No melanin
<table>
<thead>
<tr>
<th>Patient</th>
<th>Latent ( N\text{-acetyl-}\beta\text{-glucosaminidase} ) activity (%)</th>
<th>Acid phosphatase</th>
<th>Phosphodiesterase</th>
<th>( \alpha\text{-Glucosidase} )</th>
<th>( \alpha\text{-Galactosidase} )</th>
<th>( \alpha\text{-Mannosidase} )</th>
<th>( N\text{-Acetyl-}\beta\text{-glucosaminidase} )</th>
<th>( \beta\text{-Glucuronidase} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.A.</td>
<td>45.0</td>
<td>30.1</td>
<td>1.73</td>
<td>2.46</td>
<td>0.192</td>
<td>0.303</td>
<td>8.47</td>
<td>10.7</td>
</tr>
<tr>
<td>D.C.</td>
<td>68.1</td>
<td>32.9</td>
<td>2.56</td>
<td>2.50</td>
<td>0.227</td>
<td>0.741</td>
<td>5.41</td>
<td>22.0</td>
</tr>
<tr>
<td>Control*</td>
<td>66.9±3.4</td>
<td>10.9±0.91</td>
<td>0.390±0.08</td>
<td>0.310±0.02</td>
<td>0.06±0.006</td>
<td>0.25±0.02</td>
<td>2.03±0.28</td>
<td>4.84±0.43</td>
</tr>
</tbody>
</table>

* Mean values±SEM for 37 subjects are shown (Seymour & Peters, 1977).
Liver lysosomes in Dubin–Johnson–Sprinz syndrome

![Graphs showing subcellular fractionation of postnuclear supernatant from control and DJS syndrome subjects.](image)

**Fig. 4.** Subcellular fractionation of postnuclear supernatant from a liver biopsy from the control subject and from patient D.C. with DJS syndrome. Graphs show frequency–density distribution for acid hydrolases, protein and melanin. The cross-hatched area represents, over an arbitrary abscissa interval, the amount remaining in the sample layer. Frequency is defined as the fraction of total recovered activity present in the individual fraction divided by the density span covered. The percentage recovered activities (control: DJS syndrome) are **N-acetyl-β-glucosaminidase** (78:83); **acid phosphatase** (86:95); **β-glucuronidase** (93:84); **protein**, 108; **melanin**, 84.

pigment was detectable in specimens from normal liver.

We have also shown a striking increase in all lysosomal enzyme activities measured in whole liver homogenates from these two patients with the DJS syndrome. By analogy with the congenital lysosomal storage diseases (van Hoof & Hers, 1968), this suggests that there may be an accumulation of undegradable material within lysosomes. A similar increase in lysosomal enzyme activities occurs in two other acquired lysosomal storage diseases, one associated with an accumulation of cholesterol (Peters & de Duve, 1974) and the other with the storage of iron (Peters & Seymour, 1976).

Subcellular fractionation studies of the postnuclear supernatant from liver tissue homogenate of these patients showed a marked change in the distribution of lysosomal enzymes when compared with control tissue. This abnormality was specific for the lysosomes and was not shown for other organelles including mitochondria and peroxisomes. There was no definite peak of acid hydrolase activity within the gradient and most of the activity,
like melanin, remained in the sample layer on top of the gradient. That this abnormal distribution of acid hydrolases was due to lysosomes of reduced equilibrium density, rather than lysosomal disruption, was shown by the demonstration of essentially normal lysosomal integrity as determined by assays of latent N-acetyl-β-glucosaminidase. This was confirmed by the subfraction studies on the 20 000 g pellet, which showed a similar distribution indicating that the N-acetyl-β-glucosaminidase was associated with lysosomes of low density. The low density of the lysosomes in the tissue from patients with DJS syndrome strongly suggests that they have accumulated significant amounts of lipid (Peters & de Duve, 1974).

Studies on the Corriedale sheep, shown to have a similar excretory defect to the DJS syndrome in man, have demonstrated black pigment within hepatocytes on microscopy and the accumulation of melanin pigment within lysosomes (Arias, Bernstein & Toffler, 1964; Cornelius, Arias & Osburn, 1965). Isolation of the pigment granules from affected sheep liver have confirmed the presence of melanin and tyrosine derivatives (Arias et al., 1964). The accumulation of pigments within the lysosomes together with increased activities of acid hydrolases suggests a form of lysosomal storage disease. A similar situation occurs when there is congenital deficiency of a lysosomal enzyme with the consequential accumulation of the substrate for the missing enzyme (van Hoof &
Liver lysosomes in Dubin–Johnson–Sprinz syndrome

It is unlikely that the DJS syndrome is a primary disorder in lysosomal degradation of melanin and lipofuscin. The former compound is not generally considered to be a substrate for the acid hydrolases and lipofuscin is a pigment often found within lysosomes as an age-related ‘wear and tear’ pigment (Weinbren, 1961; Porta & Hartroft, 1969). The origin of the intralysosomal pigments and the mechanism of their accumulation within this organelle is the subject of current study.

This syndrome is thus a further example of a secondary lysosomal storage disease. It is of particular interest to compare this disease with haemochromatosis, which appears to be another form of secondary lysosomal storage disease affecting the liver (Peters & Seymour, 1976). In both diseases there are increased activities of several acid hydrolases with accumulation of undegradable material within the lysosomes. However, in the DJS syndrome the lysosomal integrity is within normal limits, whereas in haemochromatosis, particularly when the iron overloading is gross, the lysosomes are extremely fragile. It is noteworthy that in the DJS syndrome overall liver function is not significantly impaired, whereas in haemochromatosis there is usually severe liver damage.

Acknowledgments

We are indebted to Professor J. P. Shillingford and Dr D. K. Peters for allowing us to study their patients, to Professor K. Weinbren for reviewing the biopsy material, to Professor I. Magnus for estimating the porphyrins and to Mr Bill Hinkes for the photomicrographs. The expert technical assistance of Mr P. J. White is gratefully acknowledged. We would also like to thank Miss Berny Murphy for typing the manuscript. This work is supported by the Medical Research Council and the Wellcome Trust.

References


**Peters, T.J. & Seymour, C.A.** (1976) Acid hydrolase activities and lysosomal integrity in liver biopsies from patients with iron overload. *Clinical Science and Molecular Medicine, 50*, 75–78.


**Seymour, C.A. & Peters, T.J.** (1977) Enzyme levels in human liver biopsies. Assay methods and activities of some lysosomal and membrane-bound enzymes in control tissue and serum. *Clinical Science and Molecular Medicine, (In press).*


